Phosphorus Use Efficiency of Bio-Based Fertilizers: Bioavailability and Fractionation

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ABSTRACT

Although to date some technologies producing bio-based phosphorus (P) fertilizers have been proposed and implemented, the efficient use of the recovered products is still limited due to legislative constraints and lack of insights in the P release with time and in the corresponding mechanisms. The aim of this work was to evaluate the fertilizer performance in terms of P release and use efficiency of recovered struvite, $FePO_4$-sludge, digestate, and animal manure as compared to fossil reserve-based mineral triple superphosphate (TSP). First, product physicochemical characteristics and P fractions in the context of European fertilizer legislation were assessed. Next, a controlled greenhouse experiment was set up to evaluate plant reactions as well as changes of P availability in a sandy soil with high P status and a Rheinsand soil with low P status. Soil P fractions were determined in the extracts with water, ammonium lactate and $CaCl_2$, and in soil solution sampled with Rhizon soil moisture samplers. Based on all results, it is worth conducting long-term field trials to evaluate the P release effect of struvite and digestate as compared to animal manure and TSP on different soil types with varying P status. These products showed promise as sustainable substitutes for conventional P fertilizers and could contribute to a more efficient use of P in agriculture. A refined classification of P application standards/recommendations in terms of soil P status, soil texture, and fertilizer characteristics, next to the crop P demand, is recommended. Moreover, the additional use of Rhizon samplers for determination of direct available P, including dissolved organic P, is proposed for better understanding and categorization of different P fertilizers in environmental and fertilizer legislations.

Key Words: chemical soil analysis, digestate, nutrient recovery, Rhizon samplers, struvite

The rapid and increasing phosphorus (P) consumption in modern agriculture has raised concerns on both its supply security (Godfray *et al.*, 2010; Elser and Bennett, 2011; Neset and Cordell, 2012; Scholz and Wellmer, 2013) and its impact on the environment (soil P accumulation, leaching, and/or eutrophication) (Syers *et al.*, 2008; Kang *et al.*, 2011; Ranatunga *et al.*, 2013). Consequently, the effective use of soil P and P-containing mineral and organic fertilizers, as well as the cradle-to-cradle recycling of P from municipal, agricultural, and other biodegradable waste sources as green renewable fertilizers with high P use efficiency (*e.g.*, slow-release granules), has become highly important (Syers *et al.*, 2008; Ma *et al.*, 2011; Schröder *et al.*, 2011; Huang *et al.*, 2012; Zhang *et al.*, 2013).

Traditional P removal processes from waste(water) streams often involve the addition of Fe or Al salts, resulting in the production of substantial quantities of Fe/AlPO$_4$-sludge (Sano *et al.*, 2012). Alternatively, in the past decades, the controlled precipitation of struvite (MgNH$_4$PO$_4$·6H$_2$O) through addition of Mg to waste flow has gained interest as a route for P recovery (Latifian *et al.*, 2012; Ryu *et al.*, 2012). Moreover, the anaerobic (co-)digestion of animal manure, sludges, organic biological food waste, and/or energy crops has proven to be an effective technology for bio-energy production and release/mineralization of nutrients, which are concentrated in the remaining digestate (Fehrenbach *et al.*, 2008). During a preceding field-scale assessment, it has been observed that the use of a formulated mixture of digestate with its liquid fraction in agriculture as a substitute for animal manure may stimulate P mobilization in the soil, thereby increasing the use efficiency of soil minerals (to be confirmed) (Vaneeckhaute *et al.*, 2013, 2014). Especially in P saturated regions (*e.g.*, Flanders, Quebec, eastern China, Italy,

northern Spain, *etc.*; MacDonald *et al.*, 2011), the extraction of P from agricultural fields is relevant for local reuse (*e.g.*, in the horticultural sector or for plants with high P demand) and/or for industrial purposes. On the other hand, although the use of liquid digestate, with high effective N:P ratio, is interesting in terms of current legislative fertilization standards, its supply of plant-available P may be insufficient, depending on crop P demand and soil P status. Hence, additional fertilization with a source of bioavailable P may be required.

It must be understood that only a small proportion (15%–20%) of the total amount of P uptake in the plant (± 2.5 kg P_2O_5 ha^{-1} d^{-1}; EFMA, 2000) is directly provided by the fertilizer applied to that crop. The remainder comes from soil reserves. Hence, there must be adequate reserves of readily available P in the soil (Syers *et al.*, 2008). The P status of European soils has been estimated by EFMA (2000). For many countries, about 25% (5%–55%) of soils test as very low and low in readily available P. Such soils require significantly more P application to increase soil reserves and thus soil fertility. On the other hand, in many countries, some 40% (15%–70%) of soils test as high and very high in readily available P. On such soils, when growing the crops that have small, inefficient root systems, but a large daily uptake of P at critical growth stages, it may be necessary to apply more P. On soils with a medium P analysis value, applications need to sustain the P status. This may require a small extra amount of P on top of that removed with the harvested crop (EFMA, 2000).

All the above clearly indicates the relevance of fundamental comparative research on the P release pattern over time of potential sustainable alternatives for chemical P fertilizers produced from natural and exhaustive phosphate rock and/or for animal manure (products with improved P use efficiency). Such an evaluation is essential to determine the agricultural potential of new fertilizers and their responsible application (Erro *et al.*, 2011). Yet, such studies are currently lacking in literature for the above-mentioned biobased products (struvite, digestate, Fe/AlPO$_4$-sludge), although their production and availability is on the rise (Vaneeckhaute, 2015).

The performance of a fertilizer can be evaluated *via* product fractionation, plant reaction analysis, and/or chemical soil analysis (Sissingh, 1971; Prummel and Sissingh, 1983; Singh *et al.*, 2005; Dekker and Postma, 2008; van Dam and Ehlert, 2008; Millier and Hooda, 2011; Wang *et al.*, 2013). P fractionation of fertilizers is, in general, based on the P solubility in solvents with different strength and selectivity (Frossard *et al.*, 2002; He *et al.*, 2004, 2007). With respect to European legislation, the most important solvents are, ranked from strong to weak: i) mineral acid, ii) neutral ammonium citrate (NAC) solution, and iii) water (EU, 2003). Next to the P solubility, the fertilizer performance is usually expressed as bioavailability indices, such as P use efficiency (PUE). It can be based on the fresh weight (FW) and dry weight (DW) biomass yield, the growth rate, the P uptake (rate), and the degree and rate in which the P status of the soil changes, as determined by chemical methods (van Dam and Ehlert, 2008). Previous studies have shown that the crop response to P fertilization gives insufficient guidance to determine the fertilizer performance (no correlation), while chemical soil analyses can be conclusive (Prummel and Sissingh, 1983; Árendás and Csathó, 2002; van Dam and Ehlert, 2008). Therefore, most studies evaluating P fertilizers to date are based on soil bioavailability indices. Soil measurements can be divided into P capacity and P intensity of the soil, based on the strength of the extraction method. The P intensity gives an indication of the total amount of inorganic P which is directly available for the plant during a short period of time, while the P capacity gives an indication of the amount of P that may be released in the long term (Dekker and Postma, 2008).

In some countries, *e.g.*, the Netherlands, Switzerland, and Norway, fertilizer recommendations are based on the P status of the soil, measured as the ammonium lactate- and water-extractable P (P$_{AL}$ and P$_{water}$, respectively). The P$_{AL}$ value is a measure of soil P capacity, whereas the P$_{water}$ value reflects a combination of soil P capacity and intensity. In the latest decade, the method of plant-available elements (PAE) has also received increased attention. It concerns a multi-element extraction with 0.01 mol L^{-1} CaCl$_2$, and hence provides a simple alternative for many extraction procedures that are currently used for single nutrient (van Erp *et al.*, 1998; Houba *et al.*, 2000; Ehlert *et al.*, 2006). With respect to CaCl$_2$-extractable P (P$_{CaCl_2}$), this measurement gives an indication of soil P intensity (Houba *et al.*, 2000). An important limitation of all these standard methods is that root formation, soil compaction, and mineralization of organic matter are not or not sufficiently accounted for (Ehlert *et al.*, 2006; Soine, 2009; Amoakwah *et al.*, 2013). Underestimations have been observed in literature, especially for the determination of direct available P (Amoakwah *et al.*, 2013; Sánchez-Alcalá *et al.*, 2014). Alternatively, the use of Rhizon soil moisture samplers (SMS) allows assessing the total amount of P

in the actual soil solution (P_{rhizon}), including dissolved organic and inorganic forms (Eijkelkamp, 2003). Besides the ease of sampling, Rhizon SMS also overcomes the disadvantages related to traditional sampling using ceramic cups, such as the exchange of (divalent) cations and P (Grossmann and Udluft, 1991). Moreover, in contrast to the above standard methods, the use of Rhizon samplers is not destructive, less laborious and time consuming, and most importantly, it does not change the composition of soil solution in the process of extracting it (Amoakwah *et al.*, 2013; Sánchez-Alcalá *et al.*, 2014).

The main aim of this study was to evaluate the fertilizer performance of bio-based recovered products (struvite, $FePO_4$-sludge, and digestate from co-digestion) and pig manure as compared to fossil reserve-based mineral fertilizer, triple superphosphate (TSP, $Ca(H_2PO_4)_2 \cdot H_2O$). After product physicochemical analysis and P fractionation in the framework of European legislation, a controlled greenhouse experiment was set up to: i) evaluate the PUE based on plant reactions and changes in soil P bioavailability status during the most critical growing period, and ii) confirm and further study some nutrient release mechanisms previously observed under practical field conditions (Vaneeckhaute *et al.*, 2013, 2014, 2015). A second aim was to overcome the limitations of standard soil extraction methods by Rhizon SMS to determine P delivery in the short term. A controlled greenhouse experiment was preferred for this purpose so as to minimize potential soil disturbances, *e.g.*, of hydraulic levels, to which the various extraction methods are sensitive (Eijkelkamp, 2003). Based on the results, practical implications were discussed and recommendations in terms of legislative revisions and associated further field research were provided. As such, this study gave valuable information to guide further efforts to optimize P supply and minimize accumulation and eutrophication risks, aiming at a more responsible and efficient use of P in agriculture.

MATERIALS AND METHODS

Product characteristics and P fractions

TSP was collected at Triferto, Ghent, struvite at the NuReSys Water Treatment Plant of Clarebout Potatoes, Nieuwkerke-Heuvelland, and $FePO_4$-sludge at the piggery of Innova Manure, Ichtegem, all in Belgium. Animal manure was sampled at the piggery of Ivaco, Gistel, Belgium, and digestate was sampled at the SAP Eneco Energy, Houthulst, Belgium. The latter concerns a full-scale mesophilic (37 °C) anaerobic co-digestion plant (capacity: 60 kt year^{-1}, 2.83 MW$_{el}$) with an input feed consisting of animal manure (30%), energy maize (30%), and organic biological waste supplied by the food industry (40%). Two replicate samples of each waste stream were collected in polyethylene sampling bottles (10 L) and transported within 1 h to the laboratory for physicochemical analysis, carried in cooler boxes ($\pm$ 4 °C). In the laboratory, the replicate samples were stored cool (1–5 °C) for analysis after homogenization of each particular sample.

The DW content was determined as residual weight drying at 80 °C for 72 h in an oven (EU 170, Jouan SA, Saint Herblain, France). Organic carbon (OC) was determined after incineration of the dry samples for 4 h at 550 °C in a muffle furnace (Nabertherm, Lilientahl, Germany), with a conversion factor of 1.72 (Van Ranst *et al.*, 1999). Electrical conductivity (EC) and pH were determined potentiometrically using a WTW-LF537 electrode (Wissenschaftlich Technischen Werkstäten, Weilcheim, Germany) and an Orion-520A pH-meter (Orion Research, Boston, USA), respectively. The solid samples were first equilibrated for 1 h in deionized water at a 5:1 liquid to dry sample ratio and subsequently filtered through a white ribbon filter (MN 640 m, Macherey-Nagel, Düren, Germany). Total N content was determined using a Kjeltec system 1002 distilling unit (Gerhardt Vapodest, Koningswinter, Germany) after digestion of the sample in a sulphuric-salicylic acid mixture. The captured NH_3 in the distillate was then titrated with 0.01 mol L^{-1} HCl in the presence of a methyl red bromocresol green mixed indicator (Van Ranst *et al.*, 1999). The NH_4 was determined using the Kjeltec-1002 distilling unit after addition of MgO to the sample and subsequent titration (Van Ranst *et al.*, 1999). The amount of effective N for organic fertilizers was calculated from the analysis of total N and NH_4^+-N based on the official formula used for the determination of fertilizer N recommendations (personal communication in 2012 for Inagro, Beitem, Germany): Effective N = (N_{total} − NH_4^+-N) × 0.475 + NH_4^+-N × 0.8. It states that 80% of the NH_4^+-N is plant available. On top of that 47.5% of the remaining N, *i.e.*, NO_3^- and organic N, becomes plant available in the short term. Total P was determined using the colorimetric method of Scheel (1936) and Van Ranst *et al.* (1999) after wet digestion of the liquid samples using HNO_3 and H_2O_2. The absorbance at 700 nm was determined using a Jenway 6400 spectrophotometer (Barloworld Scientific T/As Jenway, Felsted, UK). Ca, Mg, and K were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES, Varian Vista MPX, Palo Alto, USA) after wet digestion in HNO_3 and H_2O_2

(Van Ranst *et al.*, 1999). The determination of the P fractions soluble in water, mineral acid (a mixture of HNO_3 and H_2SO_4), and NAC was determined as described in EU (2003).

The product characteristics are shown in Table I. The obtained data were used to calculate the maximum allowable product dosage for the different cultivation scenarios in compliance with the Flemish manure decree (MAP4, 2011).

Setup of greenhouse experiment

Soils used in the greenhouse experiment were: i) a nutrient-rich sandy soil with high P status ($P_{water} > 55$ mg P_2O_5 L^{-1} soil; Alterra, 2012) from Ranst, Belgium (pH = 5.0; EC(1:5) = 111 µS cm^{-1}; bulk density = 1.262 kg L^{-1}; oxalate extractable Fe and Al = 34 and 66 mmol kg^{-1} soil, respectively), and ii) a nutrient-poor, P-deficient ($P_{water} < 36$ mg P_2O_5 L^{-1} soil; Alterra, 2012) laboratory-grade Rheinsand soil (pH = 7.9; EC(1:5) = 67 µS cm^{-1}; bulk density = 1.612 kg L^{-1}). Methods used for soil physicochemical analysis

are described below. Although Rheinsand is rarely used for agricultural production, tests on this soil may provide additional information on the fertilizer effect itself, *i.e.*, the absolute amount of available P effectively provided by the fertilizers only. Indeed, on P-saturated soils (as is often the case in Flanders), differences in the P delivery by the fertilizers themselves may be difficult to detect due to large background concentrations. Moreover, the comparison between the high P and low P soil may provide information on the indirect P liberation from the soil complex as a result of fertilizer application. Hence, comparison with the Rheinsand soil was thought to be useful.

Plastic containers (14 cm in height and 13 cm in diameter) were filled with 1 kg of soil and the soil moisture solution was brought to field capacity (23% for sand and 19% for Rheinsand). After 2 d of equilibration (March 16, 2012), an equivalent product dose of 80 kg P_2O_5 ha^{-1} was applied to all containers (Table II). This refers to the maximum allowable yearly amount of P application to a sandy soil in Flanders

TABLE I

Physicochemical characteristics[a] of fertilizer products

Ferti-lizer	pH	EC	DW	OC	Total					NH_4^+-N	Effective N	Effective N:P_2O_5:K_2O
					P_2O_5	N	K_2O	Ca	Mg			
		mS cm^{-1}	%	% (DW)				g kg^{-1} DW				
TSP[b]	2.6	29	100	1.6 ± 0.0[c]	430 ± 5	0.49 ± 0.03	0.23 ± 0.06	0.31 ± 0.04	1.9 ± 0.3	138 ± 1	2.1 ± 0.0	0.00072:1:0.0044
Struvite	8.4	547	100	29 ± 0	293 ± 3	52 ± 2	28 ± 1	34 ± 1	11 ± 0	0.58 ± 0.00	87 ± 1	0.12:1:0.038
FePO$_4$-sludge	4.6	15	2.0 ± 0.0	25 ± 0	26 ± 1	55 ± 0	13 ± 0	30 ± 1	116 ± 5	9.5 ± 0.0	5.0 ± 0.0	1.1:1:4.5
Pig manure	7.7	35	6.2 ± 0.1	37 ± 1	53 ± 0	105 ± 0	74 ± 2	74 ± 2	74 ± 6	29 ± 0	14 ± 0	1.4:1:1.4
Digestate	8.6	37	9.8 ± 0.0	34 ± 1	29 ± 0	67 ± 0	39 ± 0	45 ± 0	58 ± 0	26 ± 0	6.1 ± 0.0	1.5:1:2.0

[a]EC = electrical conductivity; DW = dry weight; OC = organic carbon.
[b]Triple superphosphate.
[c]Means ± standard deviations (n = 2).

TABLE II

Application rates and macronutrient dosages to soil by fertilizer application at an equivalent dose of 80 kg P_2O_5 ha^{-1}

Fertilizer	Application rate	Effective N	Total[a]					OC[b]
			N	P_2O_5	K_2O	Ca	Mg	
	t DW[c] ha^{-1}				kg ha^{-1}			
TSP[d]	0.19	0.059	0.093	80	0.36	26	0.40	3.0
Struvite	0.27	9.2	14	80	3.0	0.16	24	78
FePO$_4$-sludge	3.08	92	169	80	357	29	15	770
Pig manure	1.51	112	159	80	112	44	21	559
Digestate	2.76	124	185	80	160	72	17	938

[a]Differences in N, K, Ca, and Mg application were corrected by adding the appropriate amount of 1 mol L^{-1} NH_4NO_3, K_2SO_4, $CaSO_4 \cdot 2H_2O$, and $MgSO_4 \cdot 7H_2O$ solution, respectively.
[b]Organic carbon.
[c]Dry weight.
[d]Triple superphosphate.

with the purpose of maize cultivation (MAP4, 2011).

Simultaneously, a control treatment without P was set up. Differences in N, K, Ca, and Mg application between the scenarios were corrected by adding the appropriate amount of 1 mol L^{-1} NH$_4$NO$_3$, K$_2$SO$_4$, CaSO$_4$·2H$_2$O, and MgSO$_4$·7H$_2$O solution, respectively, up to the recommendation levels of 135 kg effective N ha^{-1}, 250 kg K$_2$O ha^{-1}, 100 kg CaO ha^{-1}, and 50 kg MgO ha^{-1} and without exceeding the field capacity. Soils were homogenized and soil moisture content was again brought to field capacity with deionized water. Each treatment was repeated four times, resulting in a total of 48 containers (5 amendments and 1 control, 2 soil types, 4 replications).

After 4 d of equilibration (March 21, 2012), 7 energy maize seeds of the species Atletico (breeder: Kleinwanzlebener Saatzucht, Belgium; Food and Agricultural Organization ripeness index: 280; P demand: high) were sown in each container at a depth of 2 cm. The containers were covered with perforated plastics in order to reduce evapotranspiration. When the plants reached the height of the plastic, the plastics were removed, and the plants were thinned out to 5 plants per container. In each container a Rhizon SMS (MOM 10 cm male luer, PE/PVC tubing, 9 mL vacuette; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) was inserted diagonally from the topsoil through the soil column. A greenhouse bench at ± 20 °C was divided into 4 blocks representing the 4 replications, and in each block 12 containers were randomly placed.

The plants were lightened with Brite-Grow lamps (Lux 1500, 36 W) 50 cm above the plants in a day-night cycle (6:00 a.m.–8:00 p.m.). The soils were weighed daily and the soil moisture content was adjusted to field capacity each time. After 1 week, leakage of soil solution was visible in 2 containers: one struvite and one control treatment, both in the sandy soil. These 2 containers were removed from the experiment.

Homogeneous soil samples (10 g) were taken for analysis of P$_{AL}$, P$_{water}$, and P$_{CaCl_2}$ by means of a soil auger in the first 2 weeks and the last 2 weeks of the experiment. Rhizon soil moisture extracts were sampled weekly during the experiment and the P concentration in the soil solution as well as the pH were analyzed each time. Furthermore, the length of the plants was measured weekly. After 5 weeks of growth, the plants were harvested, their biomass yield was determined, and plant samples were taken for physicochemical analysis. The soils were maintained on the greenhouse bench and were moisturized every week up to field capacity. Finally, P$_{AL}$, P$_{water}$, and P$_{CaCl_2}$ in the

soils were measured again after 6 months.

Plant and soil analysis

The DW content of the biomass was determined as residual weight after 1 week drying at 65 °C. Macronutrients (N, P, K, Ca, and Mg) in the biomass were determined following the same methodology as described above for the product analysis.

Soil pH and EC were determined using the same procedure as described for the products. Field capacities were determined in accordance with the Compendium for Sampling and Analysis in the Flemish waste and soil remediation decree (CSA, 2012). For the determination of P$_{AL}$, 2.5 g of soil was mixed with 50 mL of ammonium lactate solution (pH 3.75), shaken for 4 h and filtered until colorless using a white ribbon filter (MN 640 m, Macherey-Nagel, Düren, Germany; CSA, 2012). For P$_{water}$, 4 g of soil and 240 mL of distilled water were mixed in a 250-mL flask, shaken for 1 h and filtered with white ribbon filter until colorless (Sissingh, 1971; EL&I, 2009). For P$_{CaCl_2}$, 1 g of dry soil was mixed with 25 mL 0.01 mol L^{-1} CaCl$_2$ in a 40-mL centrifuge tube, shaken for 1 h, centrifuged for 10 min at 4 000 r min^{-1} (Heraeus megafuge 1.0, Kendro Laboratory Products, Hanau, Germany), and filtered with white ribbon filter (Van Ranst *et al.*, 1999). Note that the P$_{CaCl_2}$ value is generally expressed as mg P kg^{-1} soil, whereas the P$_{water}$ and P$_{AL}$ values are officially expressed as mg P$_2$O$_5$ L^{-1} soil and mg P$_2$O$_5$ per 100 g soil, respectively. The P contents in the filtered extraction solutions and Rhizon SMS extracts were then determined using the colorimetric method of Scheel (1936) as mentioned above. Finally, in acidic sandy soils, P ions are expected to react with Fe and Al ions to form poorly soluble compounds (Hillel, 2008). Hence, an extraction of the soil with (NH$_4$)$_2$C$_2$O$_4$ and H$_2$C$_2$O$_4$ was also performed to determine the active forms of Fe and Al separately. The procedure described in CSA (2012) was used for this purpose.

P use efficiency (PUE)

Average PUEs (%) of the bio-based fertilizers were calculated based on plant reaction and soil status using the following equation:

$$\text{PUE(parameter)} = \frac{\text{Parameter}_{BF} - \text{Parameter}_{CK}}{\text{Parameter}_{TT} - \text{Parameter}_{CK}} \times 100 \tag{1}$$

where BF refers to the bio-based fertilizers under stu-

dy, CK to the blank treatment, and TT to the TSP treatment, and where parameter can refer to: 1) the P uptake by the plant, the plant FW and DW biomass yield, for which the PUE refers to the percentage of P in the bio-based fertilizers that has the same effectiveness as the reference P fertilizer (TSP) and 2) the P_{AL}, P_{water}, P_{CaCl_2}, and the P concentration in the soil solution extracted with Rhizon SMS (P_{rhizon}), for which the PUE refers to the increment in soil P status by application of the bio-based fertilizers as compared to the increment by application of TSP.

Statistical analysis

Statistical analyses were conducted using SAS 9.3. A one-way analysis of variance (ANOVA) was used to determine the effect of fertilizer type (*i.e.*, the independent variable, between-groups factor) on the plant and soil parameters (*i.e.*, the dependent variable) per measurement. Furthermore, a two-way ANOVA was used to determine whether any change in plant and soil parameters (*i.e.*, the dependent variable) was the result of the interaction between the fertilizer treatment (*i.e.*, the between-group factor) and time (in weeks, *i.e.*, the within-group factor). As the interaction term between time and treatment was never significant at the 5% significance level ($P = 0.05$), it was eliminated from the model. Follow-up tests were performed to determine whether the mean values for each plant and soil parameter were significantly different in time, and whether the averages of these parameters over the whole experimental period were significantly different between the treatments. The condition of normality was checked using the Kolmogorov-Smirnov test and QQ-plots, whereas equality of variances was checked with the Levene test. When homoscedascity was found, significance of effects was tested by use of an F-test and *post-hoc* pairwise comparisons were conducted using Tukey's honestly significant difference (HSD) test at $P = 0.05$. When no homoscedascity was found, a Welch F-test combined with a *post-hoc* Games-Howell test was used at $P = 0.05$. When the condition of normality was not fulfilled, the non-parametric Kruskal-Wallis test was applied instead of the one-way ANOVA. Significant parameter correlations were determined using the Pearson correlation coefficient (r).

RESULTS

Product characteristics and P fractions

First, it must be noticed that TSP and struvite were dry, granular products, while the other products were liquids. For recognition of new P fertilizers in the framework of European fertilizer legislations, the amount of P extractable in water and NAC, and the total amount of P must be demonstrated. Moreover, the amount of P soluble in mineral acid must be higher than 2% (EU, 2003). The extracted P fractions for the fertilizer products under study are shown in Table III.

TABLE III

P fractions, including total P and extractable P in water, neutral ammonium citrate (NAC), and mineral acid (MA), of fertilizer products

Fertilizer	Total P	Extractable P		
		Water	NAC	MA
		g P_2O_5 kg^{-1} $DW^{a)}$		
TSP[b)]	$430 \pm 5^{c)}$	413 ± 1	410 ± 1	398 ± 1
Struvite	293 ± 3	5 ± 0	282 ± 3	288 ± 5
$FePO_4$-sludge	26 ± 1	1 ± 0	25 ± 1	23 ± 0
Pig manure	53 ± 0	45 ± 2	48 ± 0	52 ± 0
Digestate	30 ± 0	23 ± 0	28 ± 0	30 ± 0

[a)]Dry weight.
[b)]Triple superphosphate.
[c)]Means $\pm$ standard deviations ($n = 2$).

The relative solubility of P in NAC was high for all fertilizers (91%–100% of total P), similar as the solubility in mineral acid (92%–100%). The amount of P soluble in water was low for struvite (1.7% of total P) and $FePO_4$-sludge (3.9%) as compared to TSP (96%), while their relative amount of P soluble in mineral acid was in the same line as TSP. Digestate had approximately the same P solubility in the different extraction reagents as animal manure (79%–100% of total P). Compared to TSP, the P solubility of both products in water was lower, while it was higher in mineral acid. Furthermore, the pHs of TSP and $FePO_4$-sludge were low (2.6–4.6), while those of struvite and digestate were alkaline (8.4–8.6) (Table I). The pH of pig manure was quasi neutral. Finally, all bio-based fertilizers under study added significantly more OC to the soil as compared to TSP (Table II).

Plant biomass yield and P uptake

For the sandy soil, all treatments showed a significantly higher FW biomass yield (Table IV), DW biomass yield (Table IV), and length (Fig. 1) at the harvest, as compared to the reference TSP. Conversely, the DW content and P content of the biomass were significantly higher for the TSP treatment. However, the absolute P uptake per container was only significantly higher for TSP as compared to the control (Table IV).

For Rheinsand soil, no significant differences were observed in the biomass length (Fig. 1) and DW yield (Table IV). The DW content was significantly lower for

TABLE IV

Plant fresh and dry weight (FW and DW, respectively) biomass yields and P uptake, as well as average P use efficiencies (PUE) based on the FW and DW yields and P uptake for the different fertilizer treatments in the sandy and Rheinsand soils

Soil	Treatment	FW yield	PUE(FW)	DW content	DW yield	PUE(DW)	P uptake	PUE(uptake)
		g FW kg^{-1} soil	%	g kg^{-1} plant	g DW kg^{-1} soil	%	mg P kg^{-1} soil	%
Sandy	Control	26 ± 2[a)][b)]	0	190 ± 0ab	4.7 ± 0.3a	0	32 ± 2b	0
	TSP[c)]	20 ± 2b	100	200 ± 20a	4.0 ± 0.2b	100	39 ± 3a	100
	Struvite	27 ± 0a	−21	170 ± 0bc	4.7 ± 0.1a	9.6	34 ± 2ab	22
	FePO$_4$-sludge	29 ± 1a	−68	170 ± 0c	4.9 ± 0.1a	−16	33 ± 3ab	16
	Pig manure	28 ± 1a	−46	170 ± 0bc	4.8 ± 0.2a	−9	35 ± 2ab	37
	Digestate	29 ± 1a	−67	170 ± 10c	4.8 ± 0.2a	−15	37 ± 2ab	80
	P value	0.000 1	–	0.000 1	0.000 2	–	0.012	–
Rheinsand	Control	15 ± 1b	0	260 ± 0a	3.9 ± 0.1a	0	5.4 ± 0.7c	0
	TSP[c)]	16 ± 1ab	100	250 ± 0b	4.0 ± 0.8a	100	9.2 ± 0.6a	100
	Struvite	16 ± 2ab	75	250 ± 10ab	3.9 ± 0.3a	67	7.0 ± 1.5bc	42
	FePO$_4$-sludge	17 ± 0a	159	240 ± 0b	4.1 ± 0.1a	233	5.6 ± 0.9c	3.3
	Pig manure	15 ± 1b	−8.9	260 ± 10ab	3.8 ± 0.1a	−67	8.4 ± 0.8ab	80
	Digestate	14 ± 1b	−45	270 ± 10a	3.8 ± 0.1a	−100	7.8 ± 0.5ab	63
	P value	0.003	–	0.000 1	0.2	–	0.000 1	–

[a)] Means ± standard deviations ($n = 4$).
[b)] Means followed by the same letter within each column for each soil type are not significantly different at $P < 0.05$ using *post-hoc* pairwise comparisons.
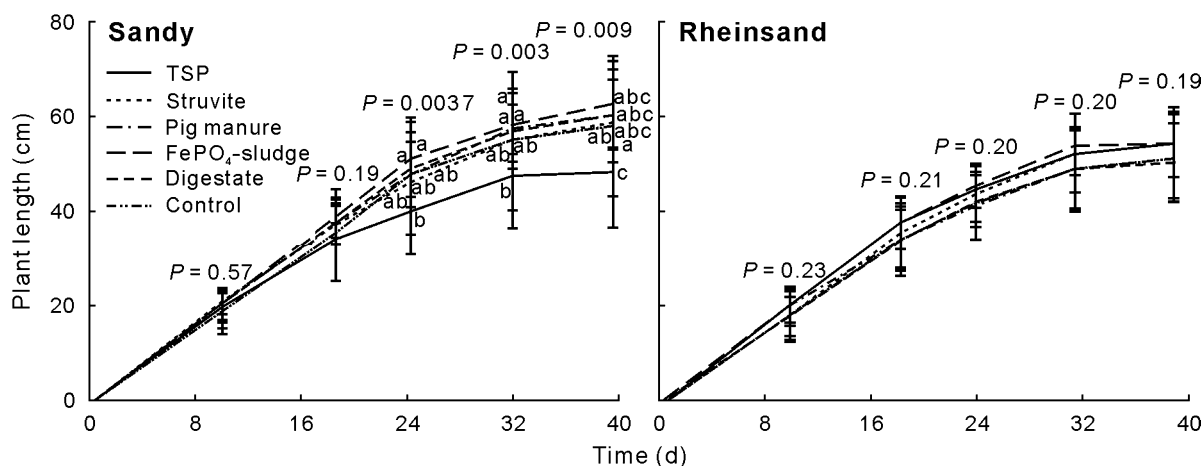[c)] Triple superphosphate.

Fig. 1   Plant length as a function of time for the different fertilizer treatments in the sandy and Rheinsand soils. Vertical bars indicate standard deviations of the means ($n = 4$). *P* values refer to the statistical analysis using one-way analysis of variance. Means with the same letter are not significantly different at $P < 0.05$ among different fertilizer treatments using *post-hoc* pairwise comparisons. TSP = triple superphosphate.

TSP and FePO$_4$-sludge as compared to the control and digestate, while FePO$_4$-sludge had a significantly higher FW yield than the control, manure, and digestate. The use of TSP, manure, and digestate resulted in a significantly higher P content in the plant and absolute P uptake as compared to the control and FePO$_4$-sludge. Moreover, the plant P uptake at the harvest was significantly lower for struvite as compared to TSP in the Rheinsand soil.

The PUEs based on the FW and DW yields, *i.e.*, PUE(FW) and PUE(DW), in the sandy soil were mostly negative as the yield of the reference TSP was lower than the control (Table IV). Among the bio-based products, the best average PUEs based on crop yield were observed for FePO$_4$-sludge and digestate, the latter simultaneously showing the highest PUE based on P uptake (PUE(uptake)). Also in the Rheinsand, the PUE(FW) and PUE(DW) were the highest for FePO$_4$-sludge, however its PUE(uptake) was the lowest. The PUE(uptake) for manure and digestate were the highest in the Rheinsand, yet their PUE(FW) and PUE(DW) were negative as the yields were slightly lower than the control (Table IV).

P_{CaCl_2}, P_{AL}, P_{water}, *and* P_{rhizon} *values*

Bioavailability curves and corresponding statistics

per measurement of P_{CaCl_2}, P_{AL} and P_{water} are presented in Fig. 2. First, it must be remarked that P_{CaCl_2} and P_{water} could only be detected in the sandy soil as the values in the Rheinsand were lower than the detection limit of both the available spectrophotometer (0.66 mg P L^{-1}; Jenway 6400, Barloworld Scientific T/As, Felsted, UK) and the continuous flow analyzer (0.05 mg P L^{-1}; AA3, Bran+Luebbe, Norderstedt, Germany).

Over the whole experimental period, the mean P_{CaCl_2} was significantly higher ($P < 0.0001$) for TSP compared to the other treatments, as well as for struvite compared to the control, digestate, and $FePO_4$-sludge (Fig. 2a). The effect of $FePO_4$-sludge on the P_{CaCl_2} value was in average significantly lower ($P < 0.0001$) than that of all other treatments. The two-way ANOVA for P_{CaCl_2} indicated a significant ($P < 0.0001$) decrease for all treatments from week 2 to 4 and week 4 to 5. The mean P_{water} values over time for TSP, digestate, and struvite were significantly higher ($P < 0.0001$) than those for the control and $FePO_4$-sludge (Fig. 2b). A significant decrease ($P = 0.0021$) was observed in week 2 for all treatments. Overall, the mean P_{AL} over time in the sandy soil was signi-

ficantly higher ($P < 0.0001$) for TSP than for all other treatments (Fig. 2c), while in the Rheinsand this effect was only significant ($P = 0.030$) compared to $FePO_4$-sludge (Fig. 2d). Both in the sandy and Rheinsand soils, no significant changes ($P > 0.1$) over time were found based on the weekly average P_{AL}. After 6 months, there was no more significant effect of the treatments on the P_{CaCl_2} ($P = 0.15$) and P_{AL} values ($P = 0.10$), whereas the control showed a significantly higher ($P = 0.0069$) P_{water} value than struvite, manure, and $FePO_4$-sludge.

The pH and P content in the soil solution extracted with Rhizon SMS (P_{rhizon}) are presented in Fig. 3. In the sandy soil, the average pH over time was significantly lower ($P < 0.0001$) for pig manure as compared to all other treatments, as well as for TSP compared to struvite, $FePO_4$-sludge, the control, and digestate (Fig. 3). Conversely, the average P_{rhizon} over time was significantly higher ($P < 0.0001$) for manure as compared to struvite, the control, and $FePO_4$-sludge (Fig. 3). The latest showed a significantly lower ($P < 0.0001$) P_{rhizon} value than the other treatments, while digestate showed a significantly higher ($P < 0.0001$) average pH than all other treatments,
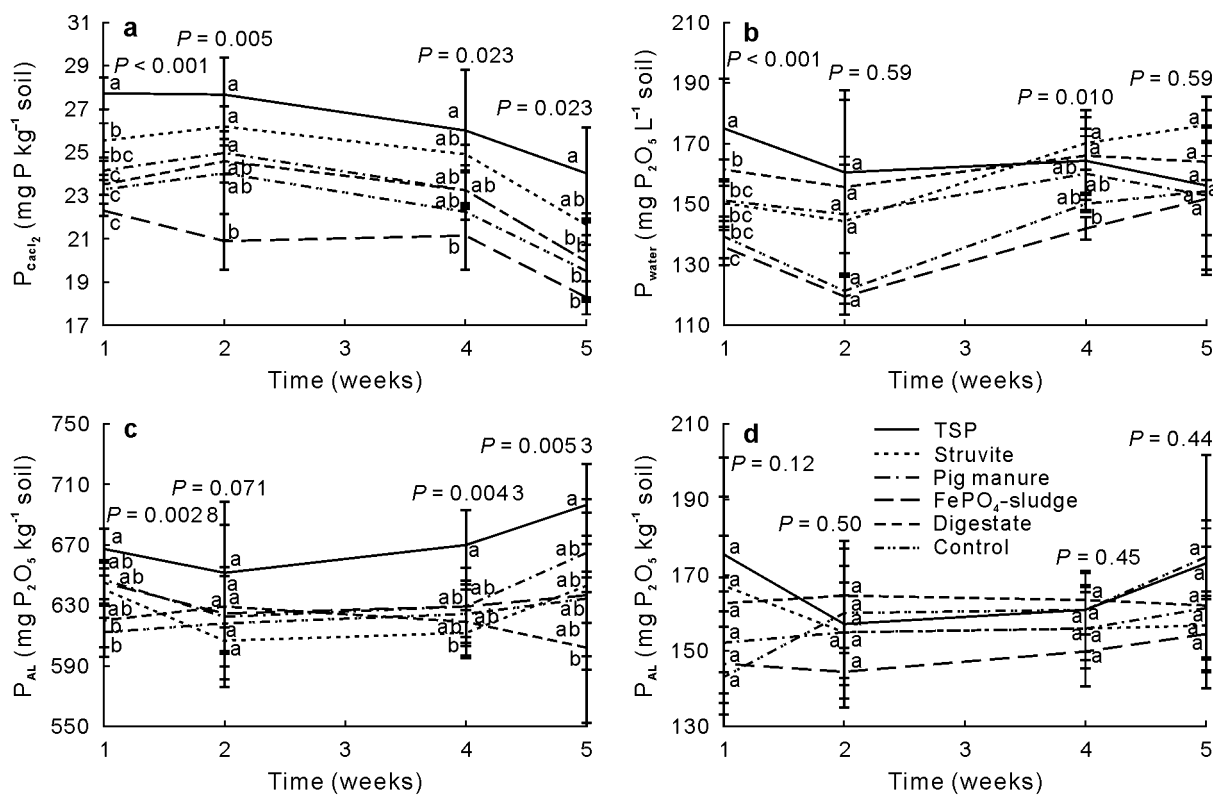

Fig. 2 $CaCl_2$-, water-, and ammonium lactate-extractable P (P_{CaCl_2}, P_{water}, and P_{AL}, respectively) in the sandy soil (a, b, and c, respectively) and P_{AL} in the Rheinsand soil (d) as a function of time after sowing for the different fertilizer treatments. Vertical bars indicate standard deviations of the means ($n = 4$). P values refer to the statistical analysis using one-way analysis of variance. Means with the same letter(s) are not significantly different at $P < 0.05$ among different fertilizer treatments using *post-hoc* pairwise comparisons. TSP = triple superphosphate.
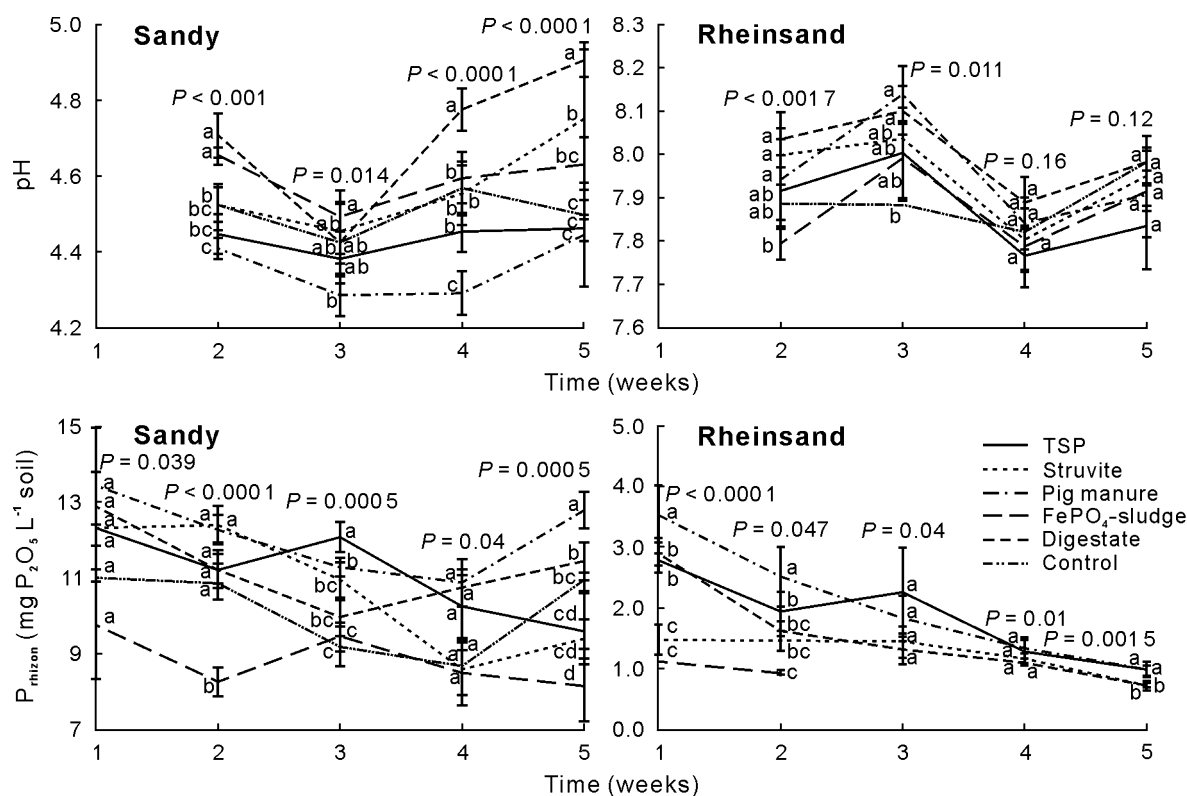
Fig. 3   pH and P concentration in the soil solution, extracted with Rhizon soil moisture samplers (P_{rhizon}), as a function of time after sowing in the sandy and Rheinsand soils. Vertical bars indicate standard deviations of the means ($n = 4$). P values refer to the statistical analysis using one-way analysis of variance. Means with the same letter(s) are not significantly different at $P < 0.05$ among the different fertilizer treatments using *post-hoc* pairwise comparisons. Detection limit = 0.05 mg P_2O_5 L^{-1}. TSP = triple superphosphate.

both in the sandy and Rheinsand soils (Fig. 3).

The average PUEs based on the various soil P analyses, *i.e.*, PUE(P_{CaCl_2}), PUE(P_{AL}), PUE(P_{water}), and PUE(P_{rhizon}), are presented in Fig. 4 as a function of time. In the sandy soil, all fertilizers presented a lower PUE(P_{CaCl_2}) and PUE(P_{AL}) than the reference TSP during the whole experimental period (Fig. 4a, c). Struvite showed the highest PUE(P_{CaCl_2}), while the P_{CaCl_2} value for FePO$_4$-sludge was even lower than the control. PUE(P_{water}) increased over time for struvite and digestate relative to TSP (Fig. 4b) and for FePO$_4$-sludge, it was negative and decreasing. PUE(P_{rhizon}) was very high (up to $> 100\%$) for pig manure in both sandy and Rheinsand soils (Fig. 4e, f). For the sandy soil, the curve for struvite showed a similar pattern as for pig manure up to week 3 (Fig. 4e). However, for the Rheinsand the values for struvite were always lower as compared to the reference and pig manure (Fig. 4f).

DISCUSSION

Plant biomass yield and P uptake

The PUE based on the plant reaction can be expressed in terms of the plant biomass yield and P up-

take. However, as the plant yield is mainly influenced by the N effect, as well as multiple other factors, such as micronutrient availability, the effect of P is hard to detect, especially for complex organic P fertilizers. Therefore, in literature the PUE based on the plant reaction is mostly calculated in terms of P uptake, if it is calculated at all (Mohanty *et al.*, 2006; van Dam and Ehlert, 2008). Indeed, some authors showed that there is no correlation between the crop response and the P supply (Prummel and Sissingh, 1983; Árendás and Csathó, 2002; van Dam and Ehlert, 2008).

Also in the present study, there was no relevant effect of the fertilizer type on the biomass yield. For the acidic sandy soil, this is not surprising due to the high P status ($P_{water} > 55$ mg P_2O_5 L^{-1} soil) of Flemish soils, *i.e.*, no response to P in terms of plant growth is expected. An odd observation was that in this high-P sandy soil, the use of the reference TSP resulted in lower biomass yields and plant lengths as compared to all other treatments under study (Table IV, Fig. 1). A similar effect was observed in the studies of Mohanty *et al.* (2006), Meena *et al.* (2007), van Dam and Ehlert (2008), Liu *et al.* (2011), and Uddin *et al.* (2012). These authors attributed this phenomenon to the fact that
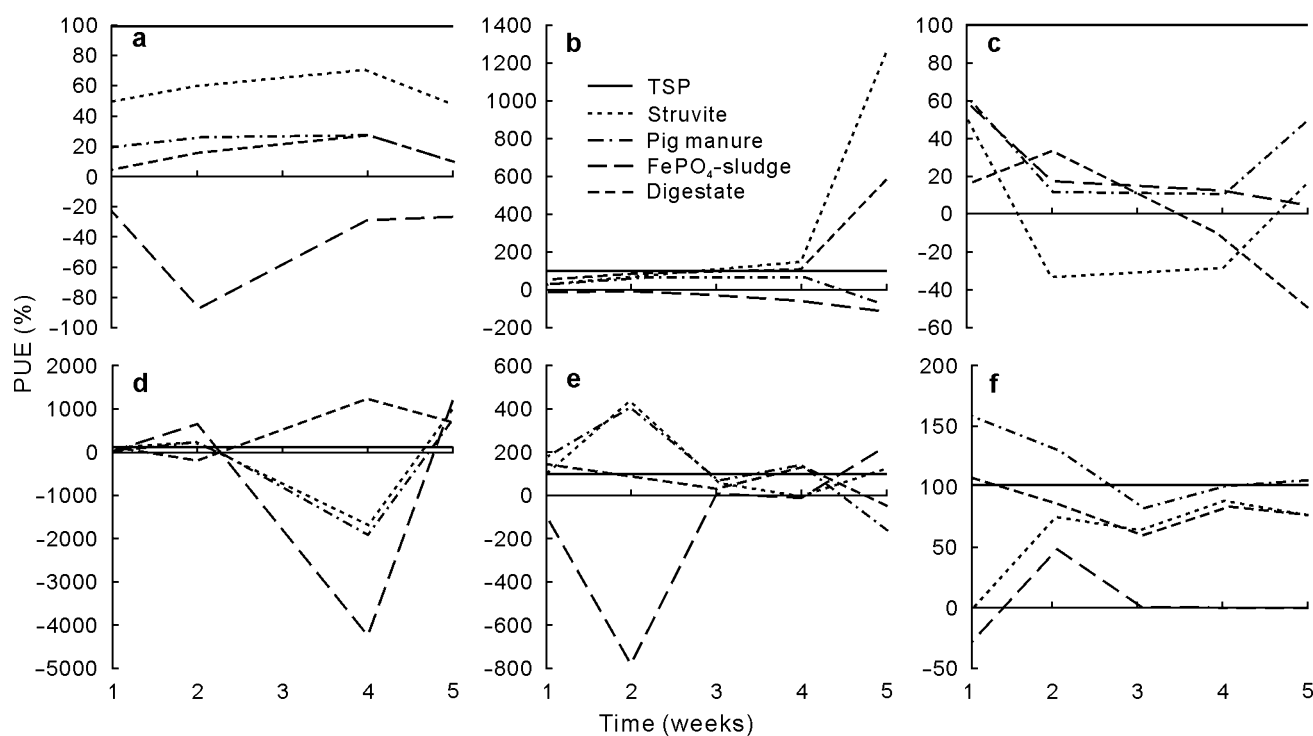
Fig. 4   Average P use efficiency (PUE) as a function of time after sowing for the different fertilizer treatments relative to the reference triple superphosphate (TSP, PUE = 100%), based on $CaCl_2$-, water-, and ammonium lactate-extractable P (P_{AL}) in the sandy soil (a, b, and c, respectively), P_{AL} in the Rheinsand soil (d), and extractable P with Rhizon soil moisture samplers in the sandy (e) and Rheinsand soils (f). TSP = triple superphosphate.

most of the P contained in TSP is water-soluble (96% in this study) and therefore partly captured by the substantial amount of Fe and Al oxides (34 and 66 mmol kg^{-1}, respectively) in acidic sandy soils. By means of a literature review, van Dam and Ehlert (2008) showed that the relative efficiency in terms of plant yield for animal manure as compared to TSP can vary between 30% and 378% (140% in this study). The higher values were, indeed, related to the conditions that hinder the operation of the readily soluble P fertilizer reference, such as phosphate fixation by Fe and Al compounds and precipitation with Ca compounds. In the Rheinsand, which had a low P level (P_{water} < 36 mg P_2O_5 L^{-1} soil), the highest FW biomass yields were obtained for TSP and $FePO_4$-sludge (Table IV). Hence, the above problem did not (or less) occur in this case.

As mentioned above, a more relevant comparison of the fertilizer effect may be made based on plant P uptake. The P uptake was the highest for TSP both in the sandy and Rheinsand soils. Yet, for the P-rich soil all amendments could cover the crop P demand (no significant difference with TSP), while for the P-poor soil $FePO_4$-sludge and struvite showed a significantly lower P uptake as compared to TSP. This indicates that the initial soil P status plays an important role in determining the plant P availability and uptake. The use

of pig manure and digestate resulted in a plant P uptake comparable to TSP in the P deficient soil, indicating that the absolute fertilizer effect in terms of direct available P is similar. The application of $FePO_4$-sludge resulted in the lowest P uptake (Table IV), indicating that the P in $FePO_4$-sludge is most fixed. This can be attributed to the stronger binding capacity of Fe and P as compared to Ca/Mg and P (Zumdahl, 2005; Hillel, 2008). In general, it can be stated that the fertilizers with the highest P solubility in water (*i.e.*, TSP, manure, and digestate) resulted in the highest plant P uptake.

Soil P availability

Struvite.   The P solubility of struvite in water was much lower as compared to the reference TSP, whereas the solubility in neutral ammonium citrate and mineral acid was relatively high (Table III), in line with literature findings (Bridger *et al.*, 1962; Barak and Stafford, 2006). These measurements indicate that struvite has slow-release properties. This was confirmed by the bioavailability curve for P_{rhizon} in the P deficient Rheinsand soil (Fig. 4f), which showed an increase in direct available soluble P from 0% to 75% as compared to TSP in approximately 2 weeks time. Moreover, it is in line with the slow-release properties

of this product for NH_4^+-N found in literature (Shu *et al.*, 2006; WERF, 2010; Latifian *et al.*, 2012; Ryu *et al.*, 2012).

In spite of these findings, struvite demonstrated the highest efficiency (relative to TSP) among the bio-based fertilizers in terms of direct available P in the P-rich sandy soil (Fig. 4a, e). This was confirmed by the significant correlation ($r = 0.63$, $P = 0.030$) between the P_{CaCl_2} for struvite and TSP in the sandy soil. The high $PUE(P_{CaCl_2})$ and $PUE(P_{rhizon})$ in the sandy soil may be attributed to the higher amount of NH_4^+-N relative to P_2O_5 in struvite (Table I). In the study of Bridger *et al.* (1962) on struvite and other metal ammonium phosphates, P release appeared to be largely the result of microbial nitrification of the ammonium constituent rather than simple dissolution. The uptake of NH_4^+ by the roots as well as the nitrification of NH_4^+ into NO_3^- are acidifying processes, which can increase soil P mobilization and uptake in the rhizosphere (Bridger *et al.*, 1962; El Diwani *et al.*, 2007). Indeed, during the first 3 weeks of growth in the struvite-amended soils, the pH in the soil solution was the lowest (Fig. 3a), while the amount of direct available P was the highest (Fig. 3c). Note that a similar effect on soil P bioavailability was found when applying bio-based recovered ammonium sulfate from acidic air scrubbing during the field trials performed by Vaneeckhaute *et al.* (2015). Other contributing factors to the extra soil P liberation could be the presence of Mg in struvite (Gonzalez-Ponce *et al.*, 2009; Ryu *et al.*, 2012) and/or its high salt content (Hartzell *et al.*, 2010).

At the end of the growing season, $PUE(P_{AL})$ and especially $PUE(P_{water})$ increased (Fig. 3b, c, d), indicating that struvite addition increased soil P reserves, mainly the readily available pool, for release in the longer term. As the plant P uptake was significantly lower for struvite than for TSP in the Rheinsand soil after 5 weeks of growth, it is likely that the release and plant uptake of P directly provided by struvite application were not yet completed at the moment of harvest. In the high P sandy soil, there was no significant difference in plant P uptake between struvite and TSP, indicating that the amount of P liberated from the soil was sufficient to support the crop demand. This difference in soil P uptake between P-rich sandy and P-poor Rheinsand again confirmed the short-term soil P mobilization provoked by struvite application, as seen above. Further research on P delivery and plant P uptake (and the corresponding mechanisms) in the longer term in different soil types with varying P status is advised for this product.

FePO_4 -sludge. FePO$_4$-sludge did not show useful as starter fertilizer for crop growth, as its P solubility in water was very low (Table III) and as were all soil bioavailability indices. In agreement to Hahn *et al.* (2002) and Nieminen *et al.* (2011), its P solubility in neutral ammonium citrate was 100%. Accordingly, the efficiency of this product to supply direct available P was low, and was the lowest among all fertilizers under study. Yet, the P capacity over time was slightly increasing, indicating that the addition of FePO$_4$-sludge slowly increased the amount of P that can be released in the longer term. Hence, the product may have slow-release properties. This phenomenon was also reflected by the highly significant correlation ($r = 0.86$, $P < 0.0001$) for P_{AL} in the sandy soil between struvite and FePO$_4$-sludge. Nevertheless, as the P_{CaCl_2} and P_{rhizon} were even lower than the control, the use of this product for agricultural crop production is discouraged, especially in P-rich soils, so as to avoid further soil P accumulation. On the other hand, the product's ability to fix P is of increasing interest for forestry and wetlands to reduce P leaching and increase P adsorption (Larsen *et al.*, 1959; Scheffer and Kuntze, 1989; Silfverberg and Hartman, 1999; Nieminen *et al.*, 2003, 2011). A long study period will, however, be required because of the slow development of active root/mycorrhiza associations that may be necessary for significant P release (Nieminen *et al.*, 2003, 2011). As such, the duration of the growth response after P fertilization is expected to be over 30 years.

Digestate and pig manure. The efficiency of digestate in supplying direct available P was slightly increasing during the greenhouse experiment (Fig. 4a), indicating that P from digestate was released slower than from the reference TSP. This product had a relatively high P solubility in water, though lower than TSP, while its P solubility in mineral acid was 100% (Table III). The $PUE(P_{water})$ was therefore high (Fig. 4b). Pig manure released direct available P somewhat faster than digestate, as the $PUE(P_{CaCl_2})$ was higher after 1 week, but equal after 4 weeks (Fig. 4a). In addition, its P solubility in water was slightly higher as compared to digestate, while the solubility in mineral acid was slightly lower (Table III). This is in line with the observed bioavailability indices: P_{CaCl_2} (Fig. 2a) and P_{rhizon} (Fig. 3c, d) were higher for pig manure than for digestate, whereas P_{water} was slightly lower (Fig. 2b).

All these results correspond to the observations made during a preceding field-scale assessment (Vaneeckhaute *et al.*, 2013, 2014, 2015) and to literature data (Güngor *et al.*, 2007; Güngor and Karthikeyan,

2008; Huang *et al.*, 2012; Möller and Müller, 2012), indicating that anaerobic (co-)digestion of animal manure reduces the fraction of direct available inorganic P in the soil solution, whereas it increases the fraction of readily available soil P that can be released in the short term. This phenomenon would be caused by the enhanced formation and precipitation of calcium phosphate, magnesium phosphate, and/or struvite through mineralization of N, P, and Mg during (co-)digestion in combination with a substantial increase of the manure pH (Le Corre *et al.*, 2009; Hjorth *et al.*, 2010; Möller and Müller, 2012). As a comprehensive example, Güngor *et al.* (2007) showed that 43% of the mineral P species in dairy manure were struvite and 57% more weakly bounded dicalcium phosphate, whereas 78% struvite and 22% hydroxylapatite were detected in the digested manure sample. It is worthy of notice that the correlation between struvite and digestate was highly significant for both P_{CaCl_2} ($r = 0.90$, $P < 0.0001$) and the pH in the soil solution ($r = 0.85$, $P < 0.0001$). Moreover, during previous field trials performed by Vaneeckhaute *et al.* (2015) a significant correlation was found between the Ca, Mg, and P use efficiency when applying digestate or its liquid fraction. Consequently, the conversion of animal manure through anaerobic (co-)digestion and the subsequent use of digestate on agricultural fields may offer a solution to control water-soluble P in soils, meanwhile supplying sufficient P to support plant growth, similar as the results observed during the previous field trials (Vaneeckhaute *et al.*, 2015).

Another interesting finding is that the P intensity of the soil, measured as P_{CaCl_2}, was lower for digestate and pig manure than for TSP (Fig. 4a), while P_{rhizon} was higher, especially for pig manure (Fig. 3c, d). It is likely that this extra amount of soluble P for the organic fertilizers, digestate and pig manure, was attributed to the release of organic P_2O_5 in the soil solution (Huang *et al.*, 2012; Roboredo, 2012), which can not (or not completely) be measured with the PAE method. Indeed, the P_{CaCl_2} values were significantly correlated ($r = 0.63$, $P < 0.0001$) for the mineral fertilizers, struvite and TSP, but no significant correlation was found between the P_{CaCl_2} measurements for the other products. On the other hand, in the Rheinsand soil, the P in the soil solution (P_{rhizon}), which includes dissolved organic forms, was significantly correlated between TSP and pig manure ($r = 0.76$, $P < 0.0001$), as well between TSP and digestate ($r = 0.73$, $P < 0.0001$), although only a relatively weak correlation was found between TSP and struvite ($r = 0.59$; $P = 0.010$). Huang *et al.* (2012) emphasized that this

organic dissolved P fraction in soils also played a role in plant P utilization. Hence, measurements carried out in the context of fertilizer recommendations and legislative standards should be able to detect both inorganic and organic P fractions.

As the average PUE(P_{rhizon}) was much higher for pig manure than for TSP both in the sandy and Rheinsand soils (Fig. 4e, f), and since pig manure is a liquid fertilizer, application of this product might cause a higher risk of leaching in the field, especially in soils low in Fe and Al (Kang *et al.*, 2011; Yang *et al.*, 2012). Since also the efficiency in terms of plant P uptake and biomass yield in the sandy soil was slightly higher (Table IV) and the soil pH significantly higher for the digestate treatment than for pig manure (Fig. 3), treating manure by anaerobic (co-)digestion before field application appears again as an interesting option from an environmental point of view. Meanwhile renewable energy can be produced. Although it is likely that similar results will be obtained for each digestion process fed with animal manure, it should be noted that the present results are based on digestate from co-digestion of pig manure (30%) with organic biological waste from the food industry (40%) and energy maize (30%).

Finally, an interesting point is that all bio-based fertilizers under study, especially digestate, added significantly more OC to the soil as compared to TSP (Table II). Application of these products could therefore also contribute to the struggle against OC depletion in many agricultural soils worldwide.

Practical implications and recommendations

In the wastewater and manure processing industry, Fe- and Al-salts are often used for P removal during solid-liquid separation so as to improve coagulation/flocculation practices and achieve water discharge levels. However, this paper indicates that the resulting $FePO_4$-sludge is not valuable for reuse as a fertilizer in terms of P release for crop growth. An increased accumulation of P in the soil is expected when using the product for agricultural purposes. Its use may be interesting on drained soils, though this remains to be evidenced. Similar results will likely be obtained for $AlPO_4$-sludge because of the comparable P binding properties of trivalent Fe and Al. However, the fertilizer effect of $AlPO_4$-sludge in terms of P bioavailability remains to be confirmed.

In the transition towards a more efficient use of nutrients in agriculture, alternative P recovery and/or release techniques are recommended instead of the traditional methods for P removal. The present paper demonstrates that manure treatment *via* anaerobic (co-)-

digestion (with other bio-degradable wastes) and/or struvite precipitation may deliver sustainable substitutes (digestate, struvite) for chemical P fertilizers and/or animal manure in agriculture. Moreover, the application of struvite may increase the liberation of P from the soil complex in high-P soils. Field-scale assessments using these bio-based products in soils with different P status are suggested to evaluate the P release and uptake in the long term, and to provide sufficient information for the establishment of responsible fertilizer application recommendations.

Although these new fertilizers are already produced and available today with quantities depending on the region (*i.e.*, legislations, nutrient excesses, *etc.*), marketing of these products also depends on the economic viability of the nutrient recovery/release technique in question and the economic competitiveness of the products as compared to commonly used fertilizers (Vaneeckhaute *et al.*, 2015). Herewith another important bottleneck arises: in many regions all derivatives produced from animal manure are currently still categorized as 'animal manure' and/or 'waste' in environmental and/or fertilizer legislation and can therefore not or only sparingly be returned to agricultural land (FAO, 2004; Lemmens *et al.*, 2007; WCC, 2015). Hence, the need exists for greater differentiation between soils, crops, and fertilizer types in the recommendations given on P fertilizer requirements (EU, 2003), as well as in fertilizer legislations. For example, in the Flemish fertilizer regulation (MAP4, 2011), currently only one standard is used for total P application as function of the crop type counts. In contrast to N standards, no distinction is made between P application from animal manure, alternative organic fertilizers, or mineral fertilizers. Moreover, the standard is currently independent of soil P status and soil texture. Only for P-saturated sandy soils one stricter norm exists, but even here no further classification is imposed.

Nevertheless, in the present study, important differences in P solubility and bioavailability for various P fertilizers and different soil P statuses were observed. Hence, a more refined legislative framework in terms of P application is advised. For this purpose, a combination of measurements of soil chemical P status, texture, and fertilizer properties (mainly P fractionation, NH_4:P ratio, and P-binding compounds as Fe, Al, Ca, and Mg), in addition to crop demand for P, is recommended. Regarding the aim to reduce P leaching and run-off, the most important parameter to evaluate is direct available P. As the PAE method does not sufficiently account for the release of dissolved organic P, measurements with Rhizon SMS are proposed as a valuable complementary method to provide the fundamental information for better categorization of different P fertilizers in environmental and fertilizer legislations. Bioavailability indices based on crop yield are generally less conclusive, hence their use is less advisable.

Besides pot experiments, the use of Rhizon SMS in field trials is possible, but the samplers have to be inserted in the soil from trenches. Trenches disturb, however, the hydraulic properties of the soil. Less mobile elements may be sampled correctly from trenches, but the concentrations of mobile nutrients may differ from those in undisturbed soil due to differing soil water conditions (Eijkelkamp, 2003). An alternative for trenches are manholes, *e.g.*, augered with an Edelman auger. Further research is recommended on the soil disturbing impact of the various sampling methods at field-scale compared to the results obtained from the controlled greenhouse experiments.

Finally, an important note is that, due to legislative constraints, the current practice of digestate processing in P-saturated regions mostly involves a solid-liquid separation step (Vaneeckhaute *et al.*, 2015). The purpose is basically to concentrate organic matter, P, Ca, and Mg in a thick fraction, which can then be pasteurized and exported (to P-poor regions). As such, the P recovery potential as concentrated and pure struvite from the liquid fraction is limited, although (local) recovery of this mineral fertilizer may be interesting and relevant, *e.g.*, for horticultural purposes or for crops with high (bio-available) P demand. Moreover, the valuable and effective organic C is eliminated from the local agricultural cycle through export (Vaneeckhaute *et al.*, 2015), while organic C depletion in many soils worldwide has become an alarming issue. This leads to the suggestion to stimulate the release of P in the liquid fraction for subsequent mineral (and pure) P recovery as struvite. As such, thick fractions with a higher C:P ratio for local reuse as soil conditioner can be recovered, and soil organic C could be maintained. Pre-treatment methods are therefore gaining importance in P-saturated regions to improve the release of P in the liquid fraction during solid-liquid separation. This will be aspect of future research.

CONCLUSIONS

Results of the greenhouse experiment indicate that some recovered P-containing bio-based products can be used as sustainable substitute for chemical P fertilizers and/or animal manure in agriculture. Struvite provided a high P availability for the plant at the beginning

of the growing season, as well as a stock for delayed slow release. The product seems to stimulate the liberation of P from soil complex, thereby providing sufficient plant available P to support plant growth in P-rich sandy soils. The addition of $FePO_4$-sludge proved not useful in terms of short-term P release. Its use as a fertilizer for agricultural crop production should be discouraged, especially in the P-rich soils. Hence, from an agronomic point of view, the implementation of struvite recovery in waste(water) treatment facilities seems more valuable than traditional practices of P removal using Fe-salts. Moreover, the P use efficiency in animal manure could be improved *via* anaerobic (co-)-digestion and application of the resulting digestate for crop production. As added benefits, negative environmental impacts of untreated animal manure are avoided, renewable energy is produced, important amounts of organic C are added to soil, and soil pH is maintained.

Furthermore, this study confirmed previous literature findings that chemical soil analyses are more conclusive than plant reaction in terms of P fertilizer performance. The additional use of Rhizon soil moisture samplers for determination of total direct available P is proposed for better understanding and categorization of different inorganic and organic P fertilizers in environmental and fertilizer legislations. This may contribute to an improved differentiation between soils, crops, and fertilizer types in the recommendations and standards given on P fertilizer requirements. Indeed, a classification of P application standards in terms of soil P status, soil texture, and fertilizer properties, next to crop P demand, is recommended. Based on the results of the presented greenhouse experiment, field-scale validation of recovered struvite and digestates as compared to animal manure and chemical P fertilizers seems worthwhile. Particular attention should be given to soil bioavailability indices, including P_{rhizon}, in various soil types with different texture and P status. This should further help to refine the P fertilizer legislations and associated recommendations.

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